

IF: 0.925

Asian Pacific Journal of Tropical Medicine

journal homepage: www.apjtm.org



doi: 10.4103/1995-7645.223532

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## Neuroprotection by misoprostol against rotenone–induced neurotoxicity in rat brain

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### ARTICLE INFO

#### Article history:

Received 19 August 2017

Received in revised form 25 October 2017

Accepted 18 November 2017

Available online 2 January 2018

#### Keywords:

Misoprostol

Rotenone

Brain oxidative stress

B cell/lymphoma-2

Paraoxonase

### ABSTRACT

**Objective:** To investigate the effect of the prostaglandin E1 analogue misoprostol on oxidative stress and neurodegeneration caused by subcutaneous rotenone administration in rats. **Methods:** Rotenone was administered in a dose of 1.5 mg/kg every other day for 2 weeks. Starting from the 1st day of rotenone injection, rats were subcutaneously treated with misoprostol at doses of 10, 100 or 1 000  $\mu$ g/kg. Rats were evaluated for brain lipid peroxidation (malondialdehyde: MDA), reduced glutathione (GSH), nitric oxide (NO) levels, and paraoxonase-1 (PON-1) activity. The concentrations of the anti-apoptotic protein B cell/lymphoma-2 (Bcl-2) were determined in the striatum. Histopathologic examination and the expression of inducible nitric oxide synthase (iNOS) in the cerebral cortex and striatum were also performed. **Results:** Compared with the vehicle-treated group, rotenone caused a significant increase in brain lipid peroxidation (MDA) by 61% ( $P<0.05$ ) accompanied by an increase in NO by 73.1% ( $P<0.05$ ) and a decrease in GSH concentration by 29.4% ( $P<0.05$ ). In addition, brain PON-1 activity significantly decreased by 63.0% ( $P<0.05$ ) and striatal Bcl-2 significantly decreased by 27.9% ( $P<0.05$ ) with respect to the corresponding control value. Brain sections from rotenone treated rats showed extensive dark pyknotic and apoptotic nuclei in neurons, shrunken cytoplasm and perineuronal vacuolation. Rotenone also caused pronounced expression of iNOS in the cerebral cortex and striatum. Treatment with misoprostol at doses of 100 and 1 000  $\mu$ g/kg resulted in decreased brain MDA (by 16.5%–23.0%) ( $P<0.05$ ) and NO levels (by 37.1%–40.7%) ( $P<0.05$ ) and increased GSH concentrations (by 18.8%–30.1%) ( $P<0.05$ ). PON-1 activity was significantly increased by 80.0%–114.8% ( $P<0.05$ ) by misoprostol at 100 and 1 000  $\mu$ g/kg, respectively. In addition, misoprostol treatment restored striatal Bcl-2 concentrations to its normal value. Misoprostol treatment resulted in markedly reduced brain injury and decreased iNOS expression in the cerebral cortex and striatum of rotenone intoxicated rats. **Conclusions:** These data suggest that misoprostol prevents the rotenone-induced neurodegeneration in rat brain by reducing brain oxidative stress.

## 1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder in which there is continued loss of the dopaminergic cells in the substantia nigra pars compacta of the basal ganglia of the mid-brain[1]. This results in dopamine depletion in the substantia nigra pars compacta dopaminergic neurons with its projections

to the striatum and the emergence of the disease symptoms such as tremor, slowness in starting movements, muscle stiffness as well as postural instability[2–4]. The cause of this cell death in PD is not well understood but biochemical processes like free radical

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**How to cite this article:** Omar M.E. Abdel-Salam, Amany A Sleem, Eman R Youness, Nadia A Mohammed, Enayat A Omara. Neuroprotection by misoprostol against rotenone-induced neurotoxicity in rat brain. Asian Pac J Trop Med 2018; 11(1): 40–47.

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mediated oxidative damage, impaired mitochondrial complex I activity, and neuroinflammation have been uncovered in the brain of PD subjects[5-9]. PD is essentially a sporadic disorder in about 95% of cases[10] and is strongly believed to be the result of exposure to an environmental toxin in addition to a genetic component[11-13]. The role of pesticides and herbicides in the pathogenesis of PD has been inferred both from epidemiological data and animal experiments[14-18]. In the latter, injection of rotenone, a pesticide of plant origin resulted in the development of motor abnormalities, brain biochemical and pathological changes similar to those found in human PD[16-18]. Rotenone inhibits mitochondrial complex I, increases reactive oxygen metabolites and evokes oxidative stress and neuroinflammatory response in brain of injected rodents[19-23]. This provided a model for understanding the disease mechanisms and finding new therapeutic targets[17,24]. Currently, there is no treatment which could prevent or even slow the process of cell loss in PD[25]. Drugs such as L-dopa, the precursor of dopamine, irreversible selective monoamine oxidase-B inhibitors, and dopamine receptor agonists aim at replacing the midbrain dopaminergic deficit by providing dopamine or boosting dopaminergic neurotransmission in the basal ganglia[26,27]. These agents help to ease the life of individuals with PD but their efficacy declines over time due to the progressive nature of the disorder together with the emergence of side effects and motor complications eg., “on-off” phenomenon, dyskinesia and dystonia[27,28]. This illustrates the need for identifying new therapies or therapeutic targets.

Misoprostol, a gastric anti-secretory agent, has been approved by the FDA for the prevention and/or treatment of gastric ulcers resulting from non-steroidal anti-inflammatory drugs[29,30]. The drug is a prostaglandin E1 (PGE1) analogue (15-deoxy-16-hydroxy-16-methyl PGE1) in which the 15-hydroxyl group of PGE1 is displaced to C16[31]. In the gastric mucosa, prostaglandins of the E series are endowed with antisecretory and cytoprotective properties[32,33]. Recent studies also indicated a neuroprotective action of misoprostol against experimental brain injury due to lipopolysaccharide endotoxin[34] or middle cerebral artery occlusion[35,36] in mice. The drug acts on prostaglandin E2 (PGE2) E-prostanoid (EP) EP2, EP3 and EP4 receptor subtypes[31,37]. These receptors have been shown to mediate an anti-inflammatory[38] and neuroprotective effects against amyloid beta-peptide[39] and the nigrostriatal toxin 6-hydroxydopamine in the brain[40]. In light of the above data, the present study aimed to investigate the neuroprotective potential of misoprostol in experimental PD caused by systemic rotenone injection in the rat.

## 2. Materials and methods

### 2.1. Animals

Male Sprague-Dawley rats (180–200 g) from the National Research Centre (Cairo, Egypt) were used. Rats were housed on a 12-h light/dark cycle and standard laboratory food and tap water freely given.

Animal procedures were done following the recommendations of the Institution Ethics Committee and that of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

### 2.2. Drugs and chemicals

Rotenone was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in dimethyl sulfoxide. Misoprostol (PGE1) was obtained from Sigma Pharmaceutical Industries (Cairo, Egypt) and dissolved in isotonic (0.9% NaCl) saline solution immediately before use. The doses selected were based on previous studies[34]. All other chemicals were of analytical grade and purchased from Sigma.

### 2.3. Study design

Rats were randomly divided into five equal groups, with six rats in each group. Group 1 received the vehicle (dimethyl sulfoxide) via the subcutaneous (s.c.) route. Group 2 received s.c. injection of rotenone at the dose of 1.5 mg/kg. Groups 3, 4 and 5 received s.c. rotenone at the dose of 1.5 mg/kg along with misoprostol at doses of 10, 100 or 1 000  $\mu$ g/kg. Drugs or vehicle were given every other day for 2 weeks. Rats were then euthanized by decapitation for tissue collection; their brains were quickly removed out on an ice-cold plate, washed with ice-cold phosphate-buffered saline (pH 7.4), weighed, and stored at 80 °C until further biochemical studies. The brain tissues were homogenized with 0.1 mol/L phosphate buffer saline at pH 7.4 to give a final concentration of 100 g/L for the biochemical assays.

### 2.4. Determination of lipid peroxidation

Lipid peroxidation was determined by the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a red colored TBA-MDA2 adduct. The absorbance was measured at 532 nm[41].

### 2.5. Determination of nitric oxide (NO)

The generation of NO was determined by measuring accumulation of nitrite using Greiss reagent as described previously. The concentration of nitrite was calculated using a standard curve for sodium nitrite. Absorption was measured at 540 nm[42].

### 2.6. Determination of reduced glutathione (GSH)

GSH was measured using the method of Ellman *et al*[43]. DTNB (5,5'-dithiobis (2-nitrobenzoic acid) or Ellman's reagent is reduced by the free sulfhydryl group on GSH molecule to yield generate 5-thio-2-nitrobenzoic acid which has yellow color and can be determined by reading absorbance at 412 nm.

### 2.7. Determination of paraoxonase-1 (PON-1) activity

Arylesterase/Paraoxonase activity was estimated in brain tissue with the use of phenyl acetate as a substrate. In this assay, phenyl acetate is hydrolyzed by PON-1 and the rate at which phenol is produced is measured using spectrophotometer by measuring absorbance at 270 nm. One unit of arylesterase activity is considered equivalent to 1  $\mu\text{mol/L}$  of phenol formed/minute. Enzyme activity is expressed in kilo International Unit/Liter (kU/L)[44,45].

### 2.8. Determination of B cell/lymphoma-2 (Bcl-2)

A commercially available human Bcl-2 enzyme-linked immunosorbent assay kit from Glory Science Co, Ltd (Del Rio, TX, USA) was used.

### 2.9. Histopathological studies

The brain tissues were immediately fixed in 10% formalin, dehydrated in gradual ethanol (50%–100%), cleared in xylene and embedded in paraffin. Sections (4  $\mu\text{m}$ ) were prepared and then stained with hematoxylin and eosin (H&E) dye for photomicroscopic observations.

### 2.10. Immunohistochemistry for iNOS

Sections were deparaffinized and treated with 0.3%  $\text{H}_2\text{O}_2$  in methanol at 37 °C for 30 min. Sections then were incubated overnight at 4 °C with primary anti-iNOS antibody (1:50 dilution) followed by incubation with the biotinylated secondary antibody and finally with avidin-horseradish peroxidase-conjugated biotin. The staining was visualized with 3,3'-diaminobenzidine. Sections were then counterstained with hematoxylin.

### 2.11. Quantitative image analysis of immunohistochemical iNOS

Leica Qwin 500 image system was used for densitometry measurement of iNOS immunoreactivity. Fifteen random fields/sections were averaged to determine means.

### 2.12. Statistical analysis

Data are presented as mean  $\pm$  SEM. One way analysis of variance and Duncan's multiple range test were used for statistical evaluation of the data. SPSS software was used for this purpose. A *P* value less than 0.05 is considered to be statistically significant.

## 3. Results

### 3.1. Oxidative stress

Rotenone caused significant increase in brain lipid peroxidation indicated by the rise in MDA level by 61.0% (*P*<0.05) compared with the vehicle control value. An increase in NO by 72.1% (*P*<0.05) was also noted in brain of rotenone-treated rats compared to the vehicle group. On the other hand, there was significant decrease in GSH by 29.4% (*P*<0.05) of its control value (Table 1).

In rotenone intoxicated rats, treatment with misoprostol at doses of 100 and 1 000  $\mu\text{g/kg}$  resulted in significant decrease in brain malondialdehyde level by 16.5%–23.0% (*P*<0.05). The content of brain NO significantly decreased by 22.0% (*P*<0.05), 37.1% (*P*<0.05) and 40.7% (*P*<0.05) in rats treated with 10, 100 and 1 000  $\mu\text{g/kg}$  of misoprostol compared with the rotenone only group. There were also increased GSH concentrations by 18.8%–30.1% (*P*<0.05) by misoprostol at 100 and 1 000  $\mu\text{g/kg}$ , respectively (Table 1).

### 3.2. PON-1 activity

A significant decrease in brain PON-1 activity by 63% (*P*<0.05) was observed in rotenone injected rats. PON-1 activity significantly increased by 80.0%–114.8% (*P*<0.05) following misoprostol treatment at 100 and 1 000  $\mu\text{g/kg}$ , respectively (Table 1).

### 3.3. Bcl-2

In rotenone-treated rats, striatal Bcl-2 concentration was significantly decreased by 27.9% (*P*<0.05) of its vehicle control value. In rats treated with misoprostol, there was a dose-dependent increase in striatal Bcl-2 concentration (Table 1).

### 3.4. Histopathological results

The brain sections from the vehicle treated control group showed normal architecture in both cortex and striatum with neurons being arranged in neat rows with abundant cytoplasm, and the nuclei are round and basophilic (Figures 1A & 2A). In the rotenone group, the most consistent findings occurring in the histological tissue sections stained with H&E were those indicating severe degenerative changes such as shrunken cytoplasm and extensively dark pyknotic and apoptotic nuclei in neurons, and perineuronal vacuolation. Also, there were leukocytic infiltration, dilated blood vessels, congested capillaries with red blood cells (Figures 1B &

**Table 1**

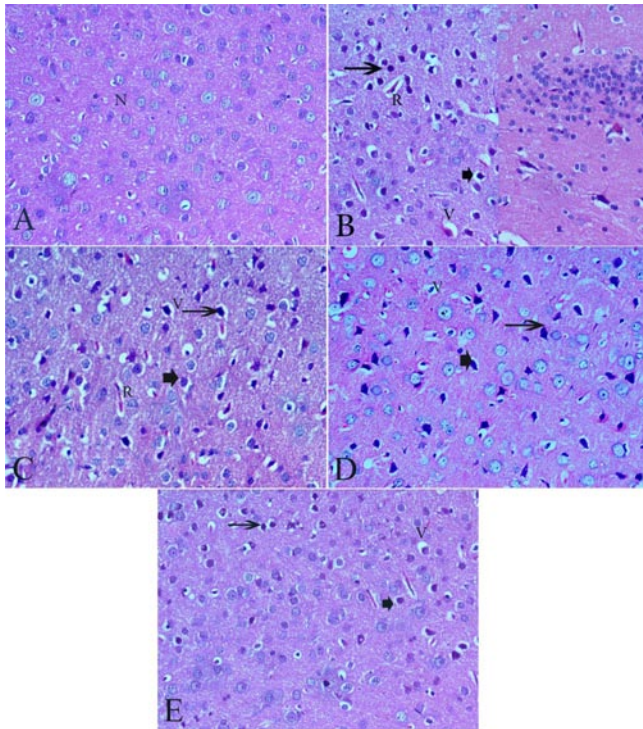
Malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), paraoxonase-1 (PON-1) activity and Bcl-2 in the brain of rats treated with rotenone alone or rotenone + misoprostol.

Group	MDA (nmol/g.tissue)	NO ( $\mu\text{mol/g.tissue}$ )	GSH ( $\mu\text{mol/g.tissue}$ )	PON1 activity (kU/L)	Bcl-2 (ng/mL)
Vehicle	23.60 $\pm$ 0.70	26.00 $\pm$ 0.61	6.54 $\pm$ 0.43	13.50 $\pm$ 0.85	3.05 $\pm$ 0.08
Rotenone	38.00 $\pm$ 1.51 <sup>a</sup>	45.00 $\pm$ 2.42 <sup>a</sup>	4.62 $\pm$ 0.17 <sup>a</sup>	5.00 $\pm$ 0.61 <sup>a</sup>	2.20 $\pm$ 0.01 <sup>a</sup>
Rotenone + misoprostol 10 $\mu\text{g/kg}$	36.00 $\pm$ 1.70 <sup>a</sup>	35.10 $\pm$ 1.68 <sup>ab</sup>	5.16 $\pm$ 0.32 <sup>a</sup>	5.65 $\pm$ 0.37 <sup>a</sup>	2.60 $\pm$ 0.05 <sup>ab</sup>
Rotenone + misoprostol 100 $\mu\text{g/kg}$	31.72 $\pm$ 1.24 <sup>ab</sup>	28.30 $\pm$ 0.95 <sup>b</sup>	5.49 $\pm$ 0.21 <sup>b</sup>	9.00 $\pm$ 0.41 <sup>abc</sup>	2.83 $\pm$ 0.04 <sup>b</sup>
Rotenone + misoprostol 1 000 $\mu\text{g/kg}$	29.26 $\pm$ 0.84 <sup>abc</sup>	26.70 $\pm$ 0.71 <sup>bc</sup>	6.01 $\pm$ 0.19 <sup>b</sup>	10.74 $\pm$ 0.68 <sup>bc</sup>	3.00 $\pm$ 0.05 <sup>bc</sup>

Values are means  $\pm$  SEM. <sup>a</sup>*P*<0.05 vs. vehicle. <sup>b</sup>*P*<0.05 vs. rotenone only. <sup>c</sup>*P*<0.05 vs. rotenone + 10  $\mu\text{g/kg}$  misoprostol.



2B). These pathological changes were ameliorated dose-dependently by treatment with misoprostol (Figures 1C–1E & 2C–2E). Sections from rats treated with 10  $\mu\text{g}/\text{kg}$  misoprostol showed less histopathological changes except for pyknosis of some neurons, perivascular vacuolation, apoptotic nuclei, and acidophilic cytoplasm (Figures 1C & 2C). Moderate improvement was seen after 100  $\mu\text{g}/\text{kg}$  misoprostol but with perivascular vacuolation, pyknotic, apoptotic nuclei in neurons (Figures 1D & 2D). Sections from rats treated with 1 000  $\mu\text{g}/\text{kg}$  misoprostol showed almost normal neuronal cells of cortex and striatum, respectively. However, few histopathological changes such as minimal vacuolation, pyknotic, apoptotic nuclei were still seen (Figures 1E & 2E).

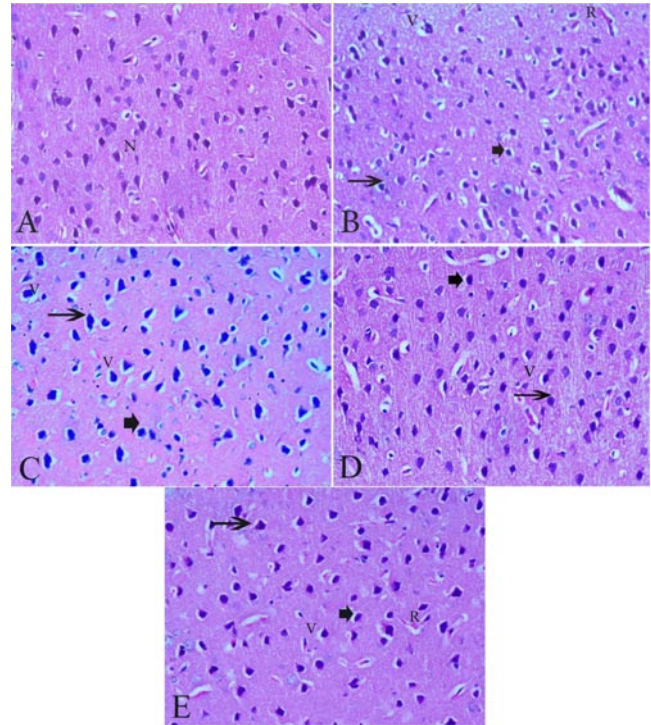


**Figure 1.** Representative light microphotographs from the cerebral cortex of mice (H & E,  $\times 400$ ).

(A) Vehicle: normal neurons (N). (B) Rotenone: shrunken cytoplasm, numerous dark pyknotic (arrow) and apoptotic (arrowhead) nuclei in neurons, surrounded by perineuronal vacuolation (V) with congested capillaries (R) and focal gliosis. (C) Rotenone + misoprostol 10  $\mu\text{g}/\text{kg}$ : still pyknotic (arrow) and apoptotic (arrowhead) nuclei, acidophilic cytoplasm, vacuolation of neuropil (V) and congested capillaries (R). (D) Rotenone + misoprostol 100  $\mu\text{g}/\text{kg}$ : few pyknotic (arrow) and apoptotic nuclei (arrowhead) with vacuolation (V). (E) Rotenone + misoprostol 1 000  $\mu\text{g}/\text{kg}$ : almost normal tissue with very few pyknotic (arrow) and apoptotic nuclei in neurons (arrowhead).

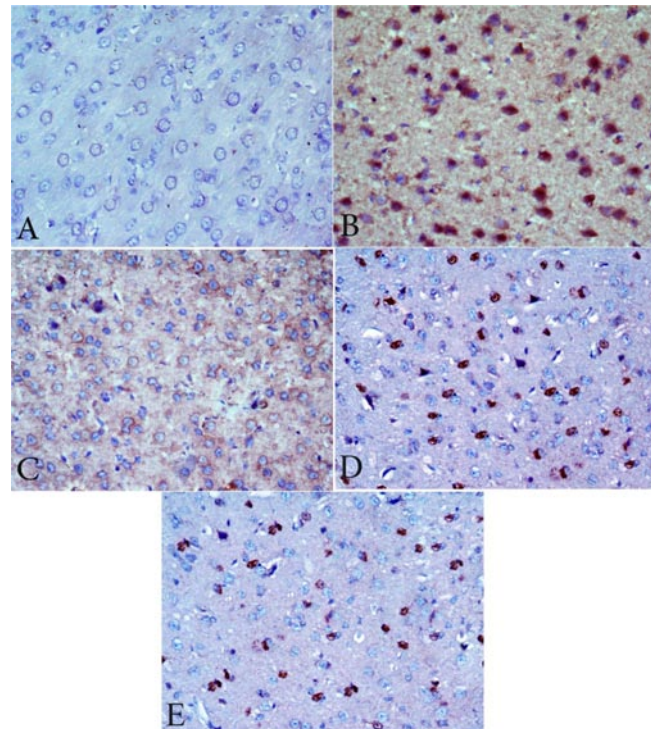
### 3.5. Immunostaining for iNOS

iNOS-positive neurons were not detected in the of cortex and striatum of the vehicle control group (Figures 3A & 4A). Rotenone caused increased density of iNOS immunoreactivity (Figures 3B & 4B). In rotenone-treated rats, misoprostol administration prevented the increase of iNOS immunoreactivity of degenerating neurons in a dose-dependent manner (Figures 3C–3E & 4C–4E).



**Figure 2.** Representative light microphotographs from the striatum of mice (H & E,  $\times 400$ ).

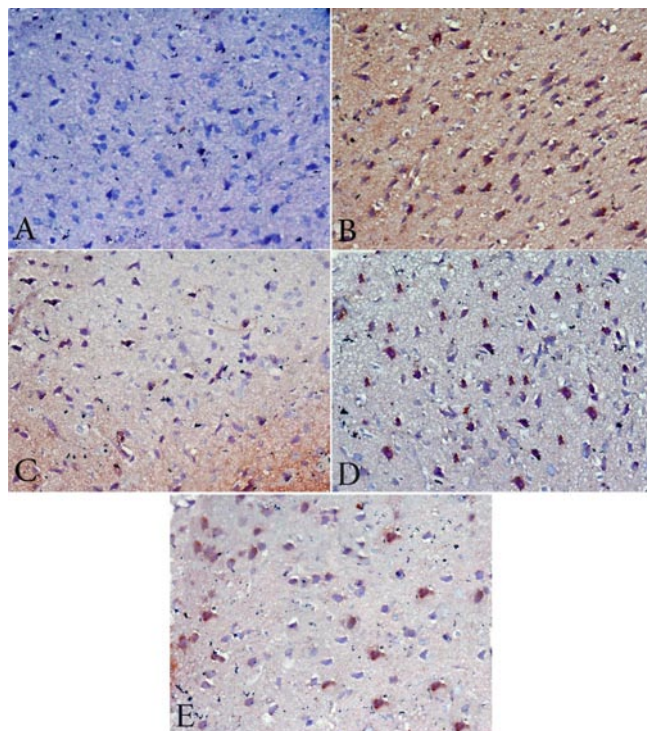
(A) Vehicle: normal neurons (N). (B) Rotenone: numerous pyknotic (arrow) and apoptotic (arrowhead) nuclei, vacuolation of neuropil (V) and congested capillaries (R). (C) Rotenone + misoprostol 10  $\mu\text{g}/\text{kg}$ : still pyknotic (arrow) and apoptotic (arrowhead) nuclei, vacuolation of neuropil (V). (D) Rotenone + misoprostol 100  $\mu\text{g}/\text{kg}$ : few pyknotic (arrow) and apoptotic neurons (arrowhead) with vacuolation (V). (E) Rotenone + misoprostol 1 000  $\mu\text{g}/\text{kg}$ : very few pyknotic (arrow), apoptotic nuclei (arrowhead) and congested capillaries (R).



**Figure 3.** Immunohistochemical microphotographs of iNOS-immunoreactivity in the cerebral cortex of mice (iNOS immunostaining with hematoxylin counterstain,  $\times 400$ ).

(A) Vehicle: no iNOS immunopositive reaction. (B) Rotenone: strong iNOS immunoreactivity. (C–E) Rotenone + misoprostol 10, 100 or 1 000  $\mu\text{g}/\text{kg}$ : decreased iNOS immunoreactivity in a dose-dependent manner.





**Figure 4.** Immunohistochemical microphotographs of iNOS-immunoreactivity in the striatum of mice (iNOS immunostaining with hematoxylin counterstain,  $\times 400$ ).

(A) Vehicle: no iNOS immunopositive reaction. (B) Rotenone: strong iNOS immunoreactivity. (C-E) Rotenone + misoprostol 10, 100 or 1 000  $\mu\text{g/kg}$ : decreased iNOS immunoreactivity in a dose-dependent manner.

### 3.6. Quantitative analysis of iNOS immunoreactivity

Quantitative morphometric analysis of iNOS immunoreactivity indicated marked increase in iNOS immunoreactivity in the cerebral cortex and striatum by rotenone compared with the vehicle-treated group. Misoprostol treatment resulted in a significant and dose-dependent decrease in immunoreactivity in these areas. iNOS immunoreactivity decreased by 38.8%, 66.0%, and 88.7% in cerebral cortex and by 37.7%, 58.0%, and 79.2% in striatum by misoprostol at doses of 10, 100, and 1 000  $\mu\text{g/kg}$ , respectively. Quantitative image analysis of iNOS immunoreactivity is shown in Table 2. These data clearly indicated that misoprostol suppressed iNOS expression in brain tissue.

**Table 2**

Optical density measurements of inducible nitric oxide synthase (iNOS) expression in the cerebral cortex and striatum of rats treated with rotenone only or rotenone + misoprostol.

Group	Optical density	
	Cerebral cortex iNOS	Striatum iNOS
Vehicle	1.92 $\pm$ 0.21	1.70 $\pm$ 0.16
Rotenone	29.75 $\pm$ 1.31 <sup>a</sup>	34.60 $\pm$ 1.25 <sup>a</sup>
Rotenone + misoprostol 10 $\mu\text{g/kg}$	18.22 $\pm$ 1.69 <sup>ab</sup>	21.54 $\pm$ 1.80 <sup>ab</sup>
Rotenone + misoprostol 100 $\mu\text{g/kg}$	10.12 $\pm$ 0.81 <sup>abc</sup>	14.52 $\pm$ 0.74 <sup>abc</sup>
Rotenone + misoprostol 1 000 $\mu\text{g/kg}$	3.35 $\pm$ 0.12 <sup>abcd</sup>	7.21 $\pm$ 1.10 <sup>abcd</sup>

Values are means  $\pm$  SEM. <sup>a</sup> $P < 0.05$  vs. vehicle-treated group. <sup>b</sup> $P < 0.05$  vs. rotenone control group. <sup>c</sup> $P < 0.05$  vs. rotenone + 10  $\mu\text{g/kg}$  misoprostol. <sup>d</sup> $P < 0.05$  vs. rotenone + 100  $\mu\text{g/kg}$  misoprostol.

## 4. Discussion

In this study we have demonstrated that the administration of the synthetic prostaglandin analogue misoprostol was able to protect against rotenone induced neurotoxicity in the rat brain. The pesticide rotenone is a mitochondrial complex-I inhibitor that is widely used in rodents to model human PD[17]. The toxicant has been shown to cause nigrostriatal damage, alpha-synuclein-like intracellular deposits, dopamine depletion and decreased tyrosine hydroxylase immunoreactivity in the substantia nigra and striatum of treated rats and mice[16,21,46,47]. In this study, rotenone administered via the subcutaneous route caused marked neuronal damage in the form of neuronal apoptosis and necrosis, and focal gliosis. The administration of rotenone was associated with markedly elevated brain oxidative stress. There was increased lipid peroxidation (MDA) along with decreased level of the antioxidant and free radical scavenging molecule GSH. These results agree with previous studies indicating increased generation of reactive oxygen metabolites by the toxicant[18,21,46-48]. Rats treated with s.c. rotenone exhibited increased MDA and decreased GSH concentrations in different brain regions[18,21,46,48-50]. Rotenone caused increased superoxide and NO in organotypic striatal slice cultures[48] and increased intracellular reactive oxygen metabolites in SH-SY5Y human neuroblastoma cell line[51]. Rotenone inhibits the mitochondrial complex I and causes increased  $\text{O}_2\cdot^-$  generation resulting in damage to complexes I and II and mitochondrial dysfunction[52]. Moreover, microglia activation by the pesticide results in increased superoxide anion production via NADPH oxidase[19] or hypochlorous acid (HOCl) via myeloperoxidase[53]. The neurotoxic effects of rotenone are largely ascribed to its ability to cause oxidative mediated cell damage. This is because the antioxidant vitamin E ( $\alpha$ -tocopherol) could prevent the rotenone effects such as protein oxidation and the decrease in tyrosine hydroxylase protein in midbrain organotypic slices[20]. In mice with orally administered rotenone, the synthetic antioxidant lipoylcarnitine prevented the increased generation of reactive oxygen metabolites in forebrain and midbrain regions and improved motor performance[54].

Our results in addition indicate markedly increased brain nitric acid content by rotenone. Moreover, immunohistochemical studies indicated that iNOS which was very rarely detected in neurons in the vehicle treated rats showed strong expression after rotenone treatment. These data are in line with other studies showing increased NO and 3-nitrotyrosine and prominent iNOS expression in different brain regions in rodents administered rotenone[21,55,56]. The increased generation of NO from iNOS during inflammatory or toxic conditions by activated astrocytes or microglia contributes to neuronal death. This is caused by the inhibition of cellular respiration and the fall in cellular ATP due to cytochrome-c oxidase inhibition and decreased activity of mitochondrial complexes (I and II)[57,58]. When present in high concentrations, NO reacts with the superoxide anion or molecular oxygen to form reactive nitrogen intermediates such as nitrogen dioxide ( $\bullet\text{NO}_2$ ), dinitrogen trioxide ( $\text{N}_2\text{O}_3$ ) or peroxynitrite radical ( $\text{ONOO}\cdot$ ) capable of amine and thiol nitrosation,

thiol oxidation, lipid peroxidation and DNA base oxidation and breakage[59]. The findings in the present study lend support to a role for NO derived from iNOS in mediating the rotenone neurotoxicity. In this context, Gao *et al.* reported attenuation of rotenone toxicity in PC12 cells *in vitro* following pretreatment with iNOS-specific inhibitor[60]. He *et al.* in addition demonstrated increased neuronal NOS expression in the striatum of rotenone-treated rats and that the neuronal NOS inhibitor 7-nitroindazole could reduce nigrostriatal damage caused by the toxicant, thereby implicating NO of neuronal origin in the rotenone neurotoxicity[61].

This study also indicated a marked decrease in PON-1 activity in the brain of rotenone intoxicated rats which is consistent with our previous observations following rotenone injection in rodents. PON-1 is a calcium-dependent esterase and plays an important role in detoxifying some organophosphate insecticides such as parathion, diazinon and chlorpyrifos[62]. It has also been shown to exhibit an anti-inflammatory and antioxidative actions[63,64]. The enzyme has been a focus of much interest because of the epidemiological data indicating that decreased activity might increase the susceptibility for developing to PD after exposure to insecticides[14,65]. The decrease in the enzyme activity after rotenone injection might be due to direct inhibition by the toxicant or is caused by the increased oxidative burden. The latter has been shown to result in inactivation of PON-1[66].

Rotenone induces apoptotic neuronal death[52,67-70]. Studies with SH-SY5Y human dopaminergic cells *in vitro* indicated that rotenone causes neuronal death via apoptosis. This involves activation of Bad, a member of the proapoptotic BH3-only subfamily of Bcl-2 proteins, caspase-9, c-Jun N-terminal protein kinase and the p38 mitogen pathways[68,69]. Rotenone also induced apoptosis in human promyelocytic leukaemia cell line (HL-60 cells) and in rat ventral mesencephalic dopaminergic neurons along with an increase in reactive oxygen metabolites, cytochrome c release and caspase-3 activation[52,67]. Protection was conferred by antioxidants or by overexpression of mangenium superoxide dismutase. This suggested the involvement of increased reactive oxygen metabolites in the rotenone-induced apoptosis[52]. In this study, there was a marked decrease in concentration of the antiapoptotic protein Bcl-2 in the striatum of rotenone intoxicated rats, which is in accordance with earlier observations[46]. The antiapoptotic protein Bcl-2 is a member of the Bcl-2 family of proteins[71]. Bcl-2 also exhibits an antioxidative action[72].

Our results clearly indicated a neuroprotective action for the PGE1 analogue misoprostol against rotenone neurotoxicity. The drug attenuated neuronal apoptosis and necrosis in the cerebral cortex and striatum and restored the level of the antiapoptotic protein Bcl-2 in the striatum. Misoprostol also reduced the oxidative stress and the expression of the inducible form of nitric oxide synthase in the brain of rotenone-treated rats. Misoprostol might thus protect against the neurotoxic action of rotenone by reducing the brain level of reactive oxygen and nitrogen species. The drug has also been shown to reduce the increase in MDA and NO and to increase

GSH concentration and PON-1 activity in the brain of mice treated with lipopolysaccharide endotoxin[34]. Misoprostol activates PGE2 EP receptor subtypes EP2 and EP3[31]. High expression of the PGE2 EP2 receptor occurs in the cerebral cortex, hippocampus and striatum[73] and mediates an anti-inflammatory and neuroprotective effect in the brain[36,38-40,73]. *In-vitro* studies show EP2 receptor activation was associated with decreased neuronal death due to glutamate toxicity or oxygen glucose deprivation[73]. In neuronal cultures, the EP2 agonist butaprost, and the EP4/EP3 agonist 1-hydroxy-PGE1 protected against neurotoxicity by amyloid beta-peptide. Stimulation of EP4/EP3 receptors was found to increase cAMP signaling and alleviate the increase in reactive oxygen metabolites[39]. In mice with focal brain ischaemia, genetic deletion of the EP2 receptor increased the extent of cerebral infarction in mice[73]. In contrast, treatment with misoprostol was shown to protect against experimental cerebral ischaemia in mice via the PGE2 EP2 and/or EP4 receptor stimulation[35,36].

In summary, the present study indicates that the synthetic PGE1 analogue and the antiulcer agent misoprostol exerted neuroprotective effects against the rotenone neurotoxicity in the rat brain. The drug shows an antioxidant and antiapoptotic actions and decreased the expression of iNOS in both the cerebral cortex and striatum. These observations suggest that misoprostol could have a place in the treatment of PD.

## Conflicts of interest statement

The authors declare that they have no conflicts of interest.

## References

- [1] Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkman J, et al. Parkinson disease. *Nat Rev Dis Primers* 2017; **3**: Article No. 17013.
- [2] Surmeier DJ, Obeso JA, Halliday GM. Selective neuronal vulnerability in Parkinson disease. *Nat Rev Neurosci* 2017; **18**(2): 101-113.
- [3] Jellinger KA. Neuropathology of nonmotor symptoms of Parkinson's disease. *Int Rev Neurobiol* 2017; **133**: 13-62.
- [4] Magrinelli F, Picelli A, Tocco P, Federico A, Roncari L, Smania N, et al. Pathophysiology of motor dysfunction in Parkinson's disease as the rationale for drug treatment and rehabilitation. *Parkinsons Dis* 2016; **2016**: 9832839.
- [5] Jiang T, Sun Q, Chen S. Oxidative stress: A major pathogenesis and potential therapeutic target of antioxidative agents in Parkinson's disease and Alzheimer's disease. *Prog Neurobiol* 2016; **147**: 1-19.
- [6] Ganguly G, Chakrabarti S, Chatterjee U, Saso L. Proteinopathy, oxidative stress and mitochondrial dysfunction: Cross talk in Alzheimer's disease and Parkinson's disease. *Drug Des Devel Ther* 2017; **11**: 797-810.
- [7] Gaki GS, Papavassiliou AG. Oxidative stress-induced signaling pathways implicated in the pathogenesis of Parkinson's disease. *Neuromolecular Med* 2014; **16**(2): 217-230.

- [8] Perier C, Vila M. Mitochondrial biology and Parkinson's disease. *Cold Spring Harb Perspect Med* 2012; **4**: a009332;
- [9] Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR. Oxidative stress and Parkinson's disease. *Front Neuroanat* 2015; **9**: 91.
- [10] Pankratz N, Foroud T. Genetics of Parkinson's disease. *Genet Med* 2007; **9**(12): 801-811.
- [11] Nandipati S, Litvan I. Environmental exposures and Parkinson's disease. *Int J Environ Res Public Health* 2016; **13**(9): 881.
- [12] Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: A review of the evidence. *Eur J Epidemiol* 2011; **26** (Suppl 1): S1-S58.
- [13] Klingenhoefer L, Reichmann H. Pathogenesis of Parkinson disease—the gut–brain axis and environmental factors. *Nat Rev Neurol* 2015; **11**: 625-636.
- [14] Baltazar MT, Dinis-Oliveira RJ, de Lourdes Bastos M, Tsatsakis AM, Duarte JA, Carvalho F. Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases—A mechanistic approach. *Toxicol Lett* 2014; **230**(2): 85-103.
- [15] Freire C, Koifman S. Pesticide exposure and Parkinson's disease: Epidemiological evidence of association. *Neurotoxicology* 2012; **33**: 947-971.
- [16] Alam M, Schmidt WJ. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. *Behav Brain Res* 2002; **136**: 317-324.
- [17] Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, et al. Mechanism of toxicity in rotenone models of Parkinson's disease. *J Neurosci* 2003; **23**: 10756-10764.
- [18] Bashkatova V, Alam M, Vanin A, Prast H, Schmidt WJ. Chronic administration of rotenone induces Parkinsonian symptoms and increases levels of nitric oxide in rat brain. *BMC Pharmacol* 2008; **8**(Suppl 1): A33.
- [19] Gao H-M, Liu B, Hong J-S. Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. *J Neurosci* 2003; **23**(15): 6181-6187.
- [20] Testa CM, Sherer TB, Greenamyre JT. Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. *Brain Res Mol Brain Res* 2005; **134**(1): 109-118.
- [21] Abdel-Salam OME, Omara EA, El-Shamarka ME-S., Hussein JS. Nigrostriatal damage after systemic rotenone and/or lipopolysaccharide and the effect of cannabis. *Comp Clin Pathol* 2014; **23**: 1343-1358.
- [22] Ding W, Xu C, Wang B, Zhang M. Rotenone attenuates renal injury in aldosterone-infused rats by inhibiting oxidative stress, mitochondrial dysfunction, and inflammasome activation. *Med Sci Monit* 2015; **21**: 3136-3143.
- [23] Zhang X, Yang Y, Du L, Zhang W, Du G. Baicalein exerts anti-neuroinflammatory effects to protect against rotenone-induced brain injury in rats. *Int Immunopharmacol* 2017; **50**: 38-47.
- [24] Abdel-Salam OME. The rotenone model of Parkinson's disease in studying the mechanisms of nigrostriatal cell death. *J Pharma Pharmacovigilance* 2015; **1**(1): 7.
- [25] Oertel W, Schulz JB. Current and experimental treatments of Parkinson disease: A guide for neuroscientists. *J Neurochem* 2016; **139**(Suppl 1): 325-337.
- [26] Tambasco N, Romoli M, Calabresi P. Levodopa in Parkinson's disease: Current status and future Developments. *Curr Neuropharmacol* 2017; doi: 10.2174/1570159X15666170510143821.
- [27] Abdel-Salam OME. Drug therapy for Parkinson's disease: An update. *World J Pharmacol* 2015; **4**(1): 117-143
- [28] Perez-Lloret S, Negre-Pages L, Damier P, Delval A, Derkinderen P, Destée A, et al. L-DOPA-induced dyskinesias, motor fluctuations and health-related quality of life: The COPARK survey. *Eur J Neurol* 2017; doi: 10.1111/ene.13466.
- [29] Rostom A, Dube C, Wells GA, Tugwell P, Welch V, Jolicoeur E, et al. Prevention of NSAID-induced gastroduodenal ulcers (review). *Cochrane Database Syst Rev* 2002; (4): CD002296.
- [30] Mejia A, Kraft WK. Acid peptic diseases: pharmacological approach to treatment. *Expert Rev Clin Pharmacol* 2009; **2**: 295-314
- [31] Woodward DF, Jones RL, Narumiya S. International Union of Basic and Clinical Pharmacology. LXXXIII: Classification of prostanoid receptors, updating 15 years of progress. *Pharmacol Rev* 2011; **63**: 471-538.
- [32] Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. *Gastroenterology* 1979; **77**: 433-443
- [33] Mózsik G. Gastric cytoprotection 30 years after its discovery by André Robert: A personal perspective. *Inflammopharmacology* 2010; **18**: 209-221
- [34] Abdel-Salam OME, Mohammed NA, Morsy SMY, Youness ER, Omara EA, Sleem AA. Misoprostol decreases oxidative stress and liver injury in bacterial lipopolysaccharide-induced endotoxemia in mice. *Comp Clin Pathol* 2014; **23**(3): 589-601.
- [35] Li J, Liang X, Wang Q, Breyer RM, McCullough L, Andreasson K. Misoprostol, an anti-ulcer agent and PGE2 receptor agonist, protects against cerebral ischemia. *Neurosci Lett* 2008; **438**(2): 210-215.
- [36] Taniguchi H, Anacker C, Suarez-Mier GB, Wang Q, Andreasson K. Function of prostaglandin E(2) EP receptors in the acute outcome of rodent hypoxic ischemic encephalopathy. *Neurosci Lett* 2011; **504**: 185-190.
- [37] Breyer RM, Bagdassarian CK, Myers SA, Breyer MD. Prostanoid receptors: Subtypes and signaling. *Annu Rev Pharmacol Toxicol* 2001; **41**: 661-690.
- [38] Shi J, Johansson J, Woodling NS, Wang Q, Montine TJ, Andreasson K. The prostaglandin E2 E-prostanoid 4 receptor exerts anti-inflammatory effects in brain innate immunity. *J Immunol* 2010; **184**(12): 7207-7218.
- [39] Echeverria V, Clerman A, Doré S. Stimulation of PGE receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following beta-amyloid exposure. *Eur J Neurosci* 2005; **22**(9): 2199-2206.
- [40] Carrasco E, Werner P, Casper D. Prostaglandin receptor EP2 protects dopaminergic neurons against 6-OHDA-mediated low oxidative stress. *Neurosci Lett* 2008; **441**(1): 44-49.
- [41] Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron induced lipid peroxidation of rat liver microsomes. *Steroids* 1994; **59**: 383-388.
- [42] Moshage H, Kok B, Huizenga JR. Nitrite and nitrate determination in



- plasma: A critical evaluation. *Clin Chem* 1995; **41**: 892-896.
- [43] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem* 1959; **82**: 70-77.
- [44] Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet* 1983; **35**(6): 1126-1138.
- [45] Haagen L, Brock A. A new automated method for phenotyping arylesterase (EC 3.1.1.2) based upon inhibition of enzymatic hydrolysis of 4-nitrophenyl acetate by phenyl acetate. *Eur J Clin Chem Clin Biochem* 1992; **30**(7): 391-395.
- [46] Abdel-Salam OME, Mohammed NA, Youness ER, Khadrawy YA, Omara EA, Sleem AA. Cerebrolysin protects against rotenone-induced oxidative stress and neurodegeneration. *J Neurorestoratol* 2014; **2**: 47-63.
- [47] Moldzio R, Piskernik, C, Radad K, Rausch W-D. Rotenone damages striatal organotypic slice culture. *Ann New York Acad Sci* 2008; **1148**: 530-535.
- [48] Ojha S, Javed H, Azimullah S, Haque ME.  $\beta$ -Caryophyllene, a phytocannabinoid attenuates oxidative stress, neuroinflammation, glial activation, and salvages dopaminergic neurons in a rat model of Parkinson disease. *Mol Cell Biochem* 2016; **418**(1-2): 59-70.
- [49] Abdel-Salam OME, Youness ER, Ahmed NA, El-Toumy SA, Souleman AMA, Shaffie B, et al. *Bougainvillea spectabilis* flowers extract protects against the rotenone-induced toxicity. *Asian Pac J Trop Med* 2017; **10**(5): 478-490.
- [50] Cui Q, Li X, Zhu H. Curcumin ameliorates dopaminergic neuronal oxidative damage via activation of the Akt/Nrf2 pathway. *Mol Med Rep* 2016; **13**(2): 1381-1388.
- [51] Liu Z, Li T, Yang D, Smith WW. Curcumin protects against rotenone-induced neurotoxicity in cell and drosophila models of Parkinson's disease. *Adv Parkinson's Dis* 2013; **2**(1): 18-27.
- [52] Li N, Ragheb K, Lawler G, Sturgis J, Rajwa B, Melendez JA, et al. Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J Biol Chem* 2003; **278**(10): 8516-8525.
- [53] Chang CY, Song MJ, Jeon SB, Yoon HJ, Lee DK, Kim IH, et al. Dual functionality of myeloperoxidase in rotenone-exposed brain-resident immune cells. *Am J Pathol* 2011; **179**(2): 964-979.
- [54] Parameshwaran K, Irwin MH, Steliou K, Pinkert CA. Protection by an antioxidant of rotenone-induced neuromotor decline, reactive oxygen species generation and cellular stress in mouse brain. *Pharmacol Biochem Behav* 2012; **101**(3): 487-492.
- [55] Xiong ZK, Lang J, Xu G, Li HY, Zhang Y, Wang L, et al. Excessive levels of nitric oxide in rat model of Parkinson's disease induced by rotenone. *Exp Ther Med* 2015; **9**(2): 553-558.
- [56] Liu C, Wang R, Ji T, Fan Y, Qin Z, Gao X. Effects of ferulic acid in rotenone induced rat model of Parkinson's disease. *J Pharmaceut Med Res* 2016; **2**(2): 55-59.
- [57] Bal-Price A, Brown GC. Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. *J Neurosci* 2001; **21**(17): 6480-6491
- [58] Wink DA, Feelisch M, Vodovotz Y, Fukuto J, Grisham MB. The chemical biology of nitric oxide. In: Gilbert and Colton, editors. *Reactive oxygen species in biological systems*. New York: Kluwer Academic/Plenum Publishers; 1999, p. 245-291.
- [59] Jiménez-Jiménez FJ, Alonso-Navarro H, Herrero MT, García-Martín E, Agúndez JA. An update on the role of nitric oxide in the neurodegenerative processes of Parkinson's disease. *Curr Med Chem* 2016; **23**(24): 2666-2679.
- [60] Gao B, Chang C, Zhou J, Zhao T, Wang C, Li C, et al. Pycnogenol protects against rotenone-induced neurotoxicity in PC12 cells through regulating NF- $\kappa$ B-iNOS signaling pathway. *DNA Cell Biol* 2015; **34**(10): 643-649.
- [61] He Y, Imam SZ, Dong Z, Jankovic J, Ali SF, Appel SH, et al. Role of nitric oxide in rotenone-induced nigro-striatal injury. *J Neurochem* 2003; **86**(6): 1338-1345.
- [62] Kulka M. A review of paraoxonase 1 properties and diagnostic applications. *Pol J Vet Sci* 2016; **19**(1): 225-232.
- [63] Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM, et al. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest* 1995; **96**: 2882-2891.
- [64] García-Heredia A, Kensicki E, Mohny RP, Rull A, Triguero I, Marsillach J, et al. PON-1 deficiency is associated with severe liver steatosis in mice fed a high-fat high-cholesterol diet: A metabolomic approach. *J Proteome Res* 2013; **12**(4): 1946-1955.
- [65] Narayan S, Liew Z, Paul K, Lee PC, Sinsheimer JS, Bronstein JM, et al. Household organophosphorus pesticide use and Parkinson's disease. *Int J Epidemiol* 2013; **42**(5): 1476-1485.
- [66] Nguyen SD, Hung ND, Cheon-Ho P, Ree KM, Dai-Eun S. Oxidative inactivation of lactonase activity of purified human paraoxonase 1 (PON1). *Biochim Biophys Acta* 2009; **1790**(3): 155-160.
- [67] Ahmadi FA, Linseman DA, Grammatopoulos TN, Jones SM, Bouchard RJ, Freed CR, et al. The pesticide rotenone induces caspase-3-mediated apoptosis in ventral mesencephalic dopaminergic neurons. *J Neurochemistry* 2003; **87**: 914-921.
- [68] Watabe M, Nakaki T. Rotenone induces apoptosis via activation of Bad in human dopaminergic SH-SY5Y cells. *J Pharmacol Exp Ther* 2004; **311**(3): 948-953.
- [69] Newhouse K, Hsuan S-L, Chang SH, Cai B, Wang Y, Xia Z. Rotenone-induced apoptosis is mediated by p38 and JNK MAP kinases in human dopaminergic SH-SY5Y cells. *Toxicol Sci* 2004; **79**: 137-146.
- [70] Qin J, Wu M, Yu S, Gao X, Zhang J, Dong X, et al. Pyrroloquinoline quinone-conferred neuroprotection in rotenone models of Parkinson's disease. *Toxicol Lett* 2015; **238**(3): 70-82.
- [71] Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, Green DR. The BCL-2 family reunion. *Mol Cell* 2010; **37**(3): 299-310.
- [72] Susnow N, Zhang L, Margineantu D, Hockenbery DM. Bcl-2 family proteins as regulators of oxidative stress. *Semin Cancer Biol*. 2009; **19**(1): 42-49.
- [73] McCullough L, Wu L, Haughey N, Liang X, Hand T, Wang Q, et al. Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. *J Neurosci* 2004; **24**(1): 257-268.